Seminar Proceeding

ILSI Southeast Asia Region conference proceedings: The gut, its microbes and health: relevance for Asia

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Background and Objectives: The human being is a complex entity, involving interaction between microbes and the human host. Evidence shows that the nutritional value of food is influenced in part by the structure and operations of an individual's gut microbial community, and food in turn shapes the individual's microbiome. A conference was held to promote understanding of the intestinal microbiome and its implications for health and disease, particularly among Asian populations. **Methods and Study Design:** Papers describing 1) the intestinal ecosystem in Asian populations, 2) changes in intestinal microbiota through life and its effects, 3) the Asian gut microbiota in disease conditions, 4) indigenous probiotics to maintain a healthy gut microbiota, 5) probiotic regulation in an Asian country, and 6) the results of a panel discussion are included in this report. **Conclusions:** The gut microbial inhabitants of Asian people differ from those of Europe and North America. Geographic location, diet, and ethnic background influence intestinal microbial composition. Urbanization and economic development have brought changes in traditional Asian diets, which in turn affected the gut microbiome, contributing to a shift in the region's health burden from infectious diseases to non-communicable chronic diseases. Novel probiotic strains of Indonesian origin demonstrated significant enhancement of humoral immune response in human studies. Knowledge gaps and implications for research to further understand the Asian gut microbiome were discussed.

Key Words: gut health, nutrition, novel probiotics, Asia, intestinal microbes

INTRODUCTION

The human intestine carries about 100 trillion microorganisms, representing hundreds of bacterial species, fungi, parasites and viruses.[1]This colonic microbiota is unique to each individual host, and changes in response to diet, drugs, disease, environmental exposure, and medical or surgical intervention. In mammals, the microbiome refers to an "extended genome" of millions of microbial genes located in the intestine.[1] Intestinal microorganisms carry out an array of enzymatic reactions that are distinct from, but critically augment, human genome-encoded activities.[2] This symbiotic condition influences human metabolism, physiology, and gene expression.[1,2]

Advances in technology and bioinformatics have made it possible to examine the influence of the intestinal ecosystem on human health. There is increasing evidencethat the nutritional value of food is influenced in part by the structure and operations of a consumer's gut microbial community, and that food in turn shapes the individual's microbiome. Changes in cultural traditions and agriculture are affecting diets worldwide, resulting in changes in the intestinal microbial composition among populations and contributing to subsequent health problems. Understanding the intestinal microbiome and approaches to its structural and functional modification will be essential for developing disease prevention strategies and personalized health care regimens.

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The objectives of the conference were to highlight current scientific knowledge on the gut microbiome, its interactions with diet and nutrition, and implications for health and disease particularly in Asia. The following papers describe the intestinal ecosystem in Asian populations, changes in intestinal microbial composition through the human life cycle and its effects, the Asian gut microbiota in disease conditions, indigenous probiotics to maintain a healthy gut microbiome and significant enhancement of humoral immune response, and probiotic regulation in an Asian country. A panel discussion at the end of the sessions is summarized. Since presentations were based on completed studies and published reports and made available online, ethics approval was not required.

RESULTS

The intestinal ecosystem

Population differences in gut microbiome in relation to diet, environment, and human genes - *Yuan Kun Lee, National University of Singapore, Singapore*

Our intestinal microbiota profile is determined by exposure during infancy, the ability of the microbe to adhere to the intestinal surface (adhesion-receptor interaction), and its ability to colonize the intestinal surface, which in turn depends on, the intestinal micro-environment as determined by host physiology stage and dietary habits. In 2009, we initiated the Asian Microbiota Program, a regional collaboration that seeks to examine how Asians differ in terms of gut microbiota. Asia is different because we cover a large geographic area and we eat very differently.

Phase 1 of the study was done among children aged 7 to 11 years. At this age, individuals largely eat at home and in the school canteen, so that foods eaten are mostly traditional and the type of food consumed is easy to check. Data were obtained from 10urban and rural cities in five countries: Japan (Tokyo & Fukuoka), China (Beijing & Lanzhou), Taiwan (Taipei & Taichung), Indonesia (Yogyakarta & Bali), Thailand (Bangkok & KhonKaen).

From the phase 1 study, we observed two distinct clusters of populations in Asia – one population with high levels of *Prevotella* and the other population with high levels of Bifidobacteria and *Bacteroides*. The family-level gut bacterial composition of children in China, Japan, and Taipei showed high levels of *Bacteroidaceae* and *Bifidobacteriaceae*. However, in Southeast Asia, except Bangkok, these same types of bacteria comprise small proportions. *Prevotellaceae* is the dominant type in Indonesia and KhonKaen. This general pattern of clustering was confirmed in a phase 2 study done among 150 adults and 128 elderly in Mongolia, Korea, China, Thailand, and Indonesia.

Random forest clustering using species-level phylotype composition data of 303 Asian children showed that there is a V-shaped clustering of microbial composition. China forms the origin of this v-shape. Taiwan and Bangkok form the two arms of the junction originating from China. KhonKaen and Indonesia cluster onto the Bangkok arm, while Japan cluster onto the Taiwan arm. As shown by their positions on two separate ends of the v-shape, Japanese and Indonesian populations have completely different gut microbiota.

The data also show that among different age groups living in the same location or in the same country, gut microbiota do not change very much except for two microbial families - *Enterobacteriaceae* and *Bifidobacteriaceae*. In China, as one gets old, *Bifidobacteriaceae* decrease. The same thing happens in Indonesia, Korea, Mongolia, Thailand. This family of bacteria are the good bacteria associated with sustaining general health and a low level is associated with many diseases. On the other hand, *Enterobacteriaceae* showed a general trend of increase as one ages, and this was observed in all countries.

What is the role of diet with respect to changes in gut microbiota structure in Asia?

What we know so far is that microbial populations differ between African and Italian children, and this has been associated with differences in diet composition. *Bacteroides* were found to be predominant among African children while *Firmicutes* were predominant among Italian children. Italian children had diets that were high in animal protein, sugar, starch and fat and low in fiber while African children had diets that were low in fat and animal protein and rich in starch, fiber and plant polysaccharides.

We also know that dietary plant products facilitate the colonization of *Prevotella* and that changes in microbiome composition are detected within 24 hours of initiating controlled feeding. Consumption of slowly digestible plant carbohydrates (oligosaccharides, wheat arabinoxylan) called prebiotics, as well as fruit, vegetables, and milk promote the growth of gut bacteria that metabolize complex carbohydrates, e.g., *Bifidobacterium* strains in particular *B.infantis*.

What is the role of meat consumption?

It has been shown that enrichment of the diet with animal protein modulates *Bacteroides* levels, with change occurring slowly within weeks accompanying pH changes in the gut rather than food per se. In a study in Mongolia, we followed changes in gut microbiota over 4 months of seasonal change. Mongolians have an interesting dietary pattern. In winter they consume large amounts of animal meat (lamb) and butter; in summer they consume mainly dairy products with little meat. Their belief is that meat has toxins and therefore shifting to dairy products during summer will reduce the burden of ingested toxins. This food pattern presents a model of dietary change twice every 6 months. Our study showed that the predominant bacterial groups - *Prevotella*, *Bacteroides*, *Ruminococcus*, *Coprococcus* - did not change regardless of diet and remained relatively constant in abundance throughout the year, suggesting that genetic contribution might play a greater role than diet, while *Faecalibacterium*, *Bifidobacterium*, *Eubacterium* changed with the seasons.

The Mongolian diet is similar to the western diet – high in meat, alcohol, fermented milk – but their gut bacterial composition differs from that of western people. The Mongolian *Firmicutes* to *Bacteroidetes* (F/B) ratio is 0.71 compared with ratios of 2.81 for urbanized Italian children and 2.95 for Koreans.

Countries that carry *Prevotella* clusters – i.e., Indonesia,

Thailand, Korea, African countries – consume relatively little meat compared with those that carry *Bacteroides* and *Bifidobacterium* clusters. The exception is Mongolia, where meat consumption is 108.8 kg per capita. Mongolian meat consumption level is very close to that of the U.S. (124.8 kg/capita)and higher than that of other *Bacteroides* clusters such as Italy, China, and Japan (90.4, 52.4, 43.9 kg/capita, respectively).

A recent paper in Nature showed that if subjects consumed solely animal products, the gut microbiota changes substantially within a short period of time. Subjects given only meat for 4 days shifted their gut microbiota from whatever microbial population was present to *Bacteroides*. The change was attributed to bile tolerance but can also be interpreted as *Bacteroides* being the default microbial population in the human intestine.

Earlier work showed that in the absence of diet, cells initiate a fucosylation program that provides food for gut *Bacteroides*. It is hypothesized that, due to an early human diet of fruits and vegetables, evolution and natural selection favoured the establishment of intestinal microbes, which break down plant products. *Bacteroides* have a complex membrane protein system that catabolizes plant cell wall glycans. Prior to being given solid foods, an infant's intestinal tract should have a good abundance of *Bacteroides* obtained from breast milk. However, studies have also shown that abundance of *Bacteroides* in advanced age is associated with chronic diseases such as cancer.

What drives Prevotella?

Prevotella is able to break down bran fiber. But bran fiber does not differentiate *Prevotella* from *Bacteroides*, as *Bacteroides* can also digest bran fiber. Something else drives *Prevotella* in Southeast Asia. One possibility is cereal, a staple food consumed in large amounts in all countries – e.g., wheat bread and potato in U.S., rice in Asia, millet and sorghum in Africa. *Prevotella* cluster countries differ from *Bifidobacteria*/*Bacteroides* countries in terms of the major staple carbohydrate foods consumed. The starch fractions of these staple foods – i.e., rapidly digestible starch, slowly digestible starch, resistant starch – show the greatest variation in resistant starch content. Can resistant starch consumption be the driving force behind *Prevotella* clusters? Does change in *Prevotella* contribute to disease? There is no doubt that geographic location dictates what products are produced, that in turn dictates diet. How much do genetic factors vis-à-vis diet contribute to gut microbiota composition among Asians? These questions pose opportunities for future work in Phase 2 of the Asian Microbiota Program.

Changes in intestinal microbiota through the human life cycle

Intestinal microbiota during life - *Patricia Conway, University of New South Wales, Australia*

The intestinal microbiota is continually evolving in terms of composition and function during life, from birth when the gut has extremely low numbers to the 100s of trillions of microbes in the adult. It is influenced by all aspects of life and in turn impacts on all aspects of life. Understanding the acquisition and development from in utero through to the adult can help identify factors which can be addressed in order to improve health and well-being as well as to slow the development of the emerging pandemic of non-communicable diseases of the modern man.

Acquisition of microbiota in the newborn

Although previously believed to be sterile at birth, we now know that low levels of colonization can occur in utero. It is reported that bacteria can transfer from the maternal gut into the placenta and amniotic fluid and the newborn can have some gut microbes. However, the greatest impact on the acquisition of microbiota includes the mode of delivery, environmental exposure including other family members, nursing staff, pets, and breast milk. Breast milk has a large population of microbes. Thus, at the time of birth and in the first few weeks of life, the baby is being exposed to microbes that in turn influence his/her individual microbiota throughout life.

Analysis of breast milk

Molecular profiling of breast milk bacteria has revealed considerable bacterial biodiversity with the composition differing between normal weight and obese mothers. There is also a significant difference in microbial composition of breast milk of mothers after vaginal delivery compared with elective/non-elective caesarean delivery (C-section). Non-elective C-section deliveries, considered as emergencies, have breast milk microbiotas that are more similar to vaginal deliveries. While the mechanisms of change have not been elucidated, it is possible that birthing stresses prior to a non-elective C-section could induce similar responses to vaginal deliveries.

Fecal bacteria of breastfed infants are similar to those in breast milk. Whereas fecal bacteria of formula-fed infants are similar to maternal fecal bacteria. The particular species of bifidobacteria in infants, *Bifidobacteria infantis*, differs from that of adults (*B. longum*). The infant strains can effectively utilize human milk oligosaccharides while the adult species are incapable of doing so. Thus breast milk contributes to the establishment of infantile intestinal microbiota by actually feeding the microbes in the infant gut and hence can be considered an important source of bionutrients.

Factors affecting the infant gut microbiota

Intrauterine contamination. There is potential for in utero contamination (translocation) of particular groups of organisms (*Bifidobacterium*, *Lactobacillus*, *Enterococcus*). The maternal microbes, antibiotic use, the mother's diet and lifestyle choices are impacting on the fetus. Babies whose mothers used probiotics prior to birth have microbes influenced by maternal exposure to dietary probiotics.

Mode of delivery. In vaginal delivery, exposure to organisms during the birth process allows the infant to establish what is considered normal gut microbes. Caesarean delivery is essentially free of exposure to bacteria, and is considered abnormal microbial introduction. It is reported that about 70% of immune sampling cells are in the intestine. Vaginal delivery leads to normal immune development since microbes initiate maturation of the intestine.

In C-section babies, delayed development of the intestinal microbes leads to abnormal and disrupted microbial colonization making these infants prone to later allergic, digestive and inflammatory conditions. Compared with C-section babies, vaginally delivered babies have very different bacterial population profiles in various regions of the body. C-section babies have elevated levels of organisms that we prefer to avoid (*Staphylococcus*, *Corynebacterium*, *Propionibacterium*) while those vaginally delivered have more favorable ones (*Lactobacillus*, *Prevotella*, *Sneathia*).

Impact of gestation time. Pre-term babies are predisposed to harmful intestinal conditions particularly necrotizing enterocolitis with increased risk of potential death. Bacterial populations differ between preterm and full term babies especially in terms of facultative anaerobes. Even at 3 months, C-section babies have not reached a picture comparable to vaginally delivered babies. Babies who develop necrotizing enterocolitis that ultimately lead to death have dramatically different intestinal microflora compared with control babies.

Type of feeding. Breastfeeding promotes increased *Bifidobacterium* levels while formula feeding promotes increased *Enterobacteriaceae* levels.

Ethnic background. Microbial composition differs by diet and ethnic background and different regions have dramatically different microbiota profiles.

Antibiotics and diet. There are significant changes in microbes present in babies that received antibiotics during the first 7 days, characterized by a reduction in microbial diversity. Use of prebiotics/probiotics promotes increased *Bifidobacterium*, *Lactobacillus*. The time of weaning and type of foods chosen also impact on the infant gut microbiota. Significant changes are found in the microbiota of colicky infants, characterized by lower diversity and slower colonization, elevated levels of *Proteobacteria* including species producing gas and inflammation, reduced lactobacilli and bifidobacteria including species with anti-inflammatory effects, and reduced beneficial butyrate-producing species.

The infant microbiota starts to resemble that of the adult by age 2 years. The impact of these influencing factors during the first 2 years will influence the health of the infant for future life. In addition to the period of birth there is a window of sensitivity at 6 months of age, when the baby is particularly susceptible to environmental challenges. Before birth, immediately after birth and during breastfeeding, the infant receives antibodies and immune protection from the mother. As breastfeeding decreases and weaning is initiated, protection from the mother's immune system on the infant is decreasing. Exposure to the environment, dirt, and change in diet are starting. This is when the baby's immune system starts to develop. The period of susceptibility coincides with the period when the mother's protection is decreasing and before the baby's immune system is completely developed, which occurs at around 6 months of age.

Succession of microbiota

Early childhood reflects increasing microbial diversity. The early microbiota is highly unstable, shifting rapidly in response to diet and illness. The 6th month and early childhood stage are therefore crucial. In healthy individuals, the microbial community during the early adult stage is highly diverse but stable and slower to react to change. Among the elderly, decreasing microbial diversity is associated with increased fragility and inflammatory markers.

One study that looked at the proportion of *Firmicutes* to *Bacteroides* in adults and the elderly revealed that change in the ratio of these two organisms correlated with change in the immune system. The elderly are predisposed to inflammatory conditions linked to change in microbiota wherein decrease in *Firmicutes* and increase in *Bacteroides* are reported and can contribute to triggering an inflammatory response.

Factors affecting the adult microbiota

Potentially whatever we do will influence the microbiota. These include lifestyle choices, medications, diet, stressors, age, institution care vs. home living, dental health, infection, hygiene, sanitation, urban/rural location. Differences in microbial diversity are linked to age and culture. Studies that looked at the distribution of different microbial groups in various populations from newborn to age 83 years showed that microbial populations of subjects from USA and Europe clustered together while other geographic regions distributed very separately from these countries.

Another study looked at the major organisms from different population groups - elderly centenarians, seniors or elderly, and young adults. Patterns were similar, but quantification of microbial groups differed. Seniors and young adults showed similar microbial profiles while that of centenarians differed dramatically. Certain bacterial groups were elevated in centenarian subjects as were pro-inflammatory cytokines.

There was also a clear distinction in microbiota depending on where the subjects lived (i.e., community vs day-hospital vs rehabilitation vs long stay care or institutionalized). Those who lived in institutions or in health service differed from young healthy controls and from those living in their own house or community. Poor health was associated with long-term care. It was concluded that microbiota composition is clustered according to residence location and the type of care received. In terms of health indices, microbial composition correlated with fragility as less diverse microbiota profiles were found in those who were more fragile, had poorer nutrition (which also tied in with dental health care because if dental health is a problem, food choice is going to differ), and detectable markers of inflammation.

We analysed the microbiota in terms of the type of bacteria that are present. I would like to emphasize that bacteria may be unchanged but they may behave differently. Some of our in vitro studies examined the change in lactobacillus using microbiota from subjects of different age and sex. Prebiotics such as fructooligosaccharides were used as substrate. Substrate utilization by fecal microbiota from a one-year-old breastfed female baby and from a 60-

yr old female were similar. But utilization of the same substrate by microbiota from a male of the same age differed. This raises the issue that we need to look at not only what's there but also what's the metabolic capacity of the host population.

To summarize microbial succession with age, during the first month of life bifidobacteria are predominant and gradually over the first year there is increase of *Bacteroides fragilis*. In the adolescent age we get contradictory results because this population is changing dramatically and is very dependent on exposures including environmental, diet and emotional factors. Biodiversity is increasing and microbial composition depends on what time samples are taken. The adult stage is mostly stable (*Firmicutes >Bacteroidetes >Proteobacteria >Actinobacteria*). The microbiota of the elderly changes (increase of *Bacteroidetes*, decrease of Bifidobacteria) with a resulting decrease in biodiversity. It's a cycle that starts off with very limited diversity and numbers and then finishes with limited biodiversity; and starting with a lot of bifidobacteria and finishing with few bifidobacteria. When we talk about human gut composition it is very important to know the age and the history of subjects. We know there are various factors impacting on the composition of gut microbiota and that this composition has an important impact on the emerging pandemic of non-communicable diseases. A better understanding of acquisition and succession of the microbiota through life will be valuable in identifying ways of improving health and wellbeing in the future.

The microbiome influences host physiology in early life- *Sven Pettersson, Karolinska Institute, Sweden/Nanyang Technological University, Singapore*

Bacteria have been around for billions of years, way before humans. Hence bacteria have had enough time to adapt and develop within the landscape which they control. In medicine, a paradigm shift has occurred, wherein humans are no longer viewed as a separate entity, but as part of a complex environment involving interaction between microbes and the human host.

The holobiont is defined as the host and its microbiota. The holobiont contains numerous genes from the host (termed the karyome), the mitochondria (chondriome), the different microbial species (microbiome, virome, fungiome), and the interactions between the organism and all other genes (epigenome). The holobiont theory sees people not just as individuals but also as ecosystems, wherein microbes and host share functions in a symbiotic fashion in order to meet their biochemical and biological needs. All genome wide association studies carried out have only looked at the host and ignored the microbiome. But changes in the host influence the microbiome and elicit a response from the microbiome. This part of the equation has not been studied.

From the holobiont perspective, microbes are part of the machinery for good metabolic regulation in all host species. There is vertical transmission of microbiota from parents to offspring, which seems to be stable, almost like genes. There is also evidence to suggest the existence of a microbiome in the placenta. Its significance is unknown. This suggests that other organs may also contain tissue-related regional microbiomes.

Pregnancy and the microbiome – effects on placenta

Pregnancy is a period of tremendous metabolic stress. The fetus has to undergo all aspects of development programming, organ development and subsequently be delivered. We used germ free (GF) mice to study what happens during pregnancy if microbes are not there. Corticosterone levels were higher in plasma of pregnant and non-pregnant GF animals compared with normal animals, indicating that GF animals are under constant stress even if they are not pregnant.

Pregnancy is associated with increased corticosterone levels because of the need for more glucose to support the developing fetus. Glucocorticoids have anti-inflammatory functions and serve as a metabolic signal to activate gluconeogenesis. If the host gets too stressed, glucocorticoids reach a certain threshold indicating insufficient amount of nutrients. This in turn induces the body to constrain programming in certain organs, including the placenta. Signalling pathways shown to be important for placental development are IGF2, PPAR beta, PPAR gamma pathways and these are regulated by glucocorticoids. When glucocorticoid levels increase, these pathways are repressed due to insufficient energy. Ongoing investigations are addressing whether placenta display morphological changes. Additional data demonstrate an increased uptake of a labelled tracer in fetus from the GF dam compared with pups from normal specific-pathogen-free (SPF) dams. These preliminary data indicate a possible increased nutritional supply to the GF pups when the maternal microbiome is not present in the GF dam.

Mechanism of microbial effects on the placenta

We examined gluconeogenesis and the enzymes which respond to elevated glucocorticoids in the liver. In GF dams, glucose was immediately absorbed and stored in tissue. It is like they have reduced glucose levels and they are trying to get more energy. GF dams have limited liver glycogen stores that are further reduced by stress. Fatty acids are absorbed in the liver and used as energy through beta-oxidation. One of the key regulators to take up fatty acids is a protein called PPAR alpha. This protein is elevated in pregnant GF animals indicating up regulation of the machinery to take up fatty acids in response to energy deficiency. Previous studies have shown that in the absence of bacteria, both GF mice and GF fish are unable to absorb lipids. Fish experiments have shown that *Firmicutes* are very efficient lipid absorbers. But while there is a big difference in plasma VLDL and triglycerides between GF animals and normal animals, liver concentrations of these compounds do not differ.

Another critical component during pregnancy is the activation of ketone bodies, which serve as an alternative source of energy. The enzymes involved in formation of ketone bodies are massively elevated in GF dams compared with SPF control dams. While the steady state level of ketone bodies in plasma of GF mice is about the same as in normal mice, the amount of ketone bodies in liver is more highly elevated in GF dams.

Maternal microbiome and offspring condition in later life
While outcomes are similar in terms of litter size and birth weight, the conditions under which offspring are generated differ between GF and normal mice. The question is - after delivery, is there an effect on how these offspring handle stress when exposed to environmental stress factors? Some studies indicate that metabolic status during pregnancy may preset conditions in later life by disrupting the HPA (hypothalamus-pituitary-adrenals) axis. Effects may include psycho-cognitive disorders, metabolic diseases such as obesity and diabetes. In an earlier study we observed that you can change behaviour in a mouse model by changing the maternal microbiome prior to pregnancy. Presumably the maternal microbiome was having an effect on development programming of the brain. It is becoming clear that what we see in the offspring in terms of susceptibility to environmental stress is set during pregnancy. Using a metabonomic approach, studies should be done in humans to understand what the maternal microbiome does. That is a target to address for the future.

The gut microbiome in health and disease – Asian studies

Undernutrition and the gut microbiome - *G. Balakrish Nair, Centre for Human Microbial Ecology, Translational Health Science and Technology Institute, India*
Microbiology is grounded on the basis of culture of microorganisms. We know now that what is cultured is only 2 to 10% of what is there and the rest (90%) are unculturable. While clinical decisions regarding diarrhea and gastrointestinal diseases are based on culture results, we know that there are a lot more microorganisms that are actually present but cannot be seen by these results.

Undernutrition and associated problems
Undernutrition is defined as the outcome of insufficient food intake and repeated infectious diseases. It includes underweight, stunting, wasting and micronutrient deficiency. Progression of undernutrition has been attributed to a variety of causal factors including inadequate dietary intake, which impairs immune responses that in turn predisposes to infectious diseases including enteric infections. This in turn alters the microbiota leading to dysregulated gut permeability causing malabsorption. An altered microbiota results in a vicious cycle of diarrhea and undernutrition in susceptible children. Undernutrition is influenced by the environment, the human genome, host nutrition and host microbiota. Its effects include impaired vaccine responses, growth failure, stunting, decreased fitness, cognitive and fluency impairment.Globally, South Asia particularly India and sub-Saharan Africa have the highest prevalence of undernutrition (i.e., stunting and wasting).

Is the gut microbiota altered in undernourished children?
In an earlier study,[3] we did face-to-face comparison of the metagenome of the gut of a malnourished child versus that of a healthy child. Compared with a healthy child, the malnourished child showed increased incidence of pathogenic members of the phylum Proteobacteria. Families *Campylobacteriaceae* and *Helicobacteriaceae* were 35- and 12-folds higher in malnourished children suggesting infection of the intestinal epithelium by pathogens belonging to these families. *Campylobacter* both *jejuni* and *coli* collectively cause campylobacteriosis, while *Helicobacter* causes peptic/duodenal ulcers and gastric cancer. Other families with higher representation in malnourished samples were *Bacteroidaceae* (found in obese individuals who lose weight rapidly), and *Porphyromonadaceae* (found in inflammatory bowel disease). In the healthy child gut metagenome, the predominance of these pathogenic lineages was not observed suggesting that the healthy flora could competitively inhibit and exclude these pathogens.

This simple study showed that there is an overall difference between microbial communities residing in the gut of the malnourished and that of the healthy child. The gut microbiota of the malnourished child is interpreted as aberrant gut microflora, a concept that extends the understanding of malnutrition beyond nutrition deprivation. Aberration of gut microflora leads to sub-clinical disorder characterized by inflammation and modest malabsorption. The continued aberration of gut microflora leads to unchecked bacterial proliferation, concurrent infection, disruption in community dynamics of commensal intestinal flora, and impaired immunity.

Gut microbiome of undernourished Indian children[4]
This study, which was published in April 2014 in PLoS One[4], was done in a district in West Bengal called Birbhum, a typical rural and agricultural setting. The Birbhum Population Project is a health and demographic surveillance system performing longitudinal observations. The study aimed to determine how undernutrition affects microbiome membership and function. Twenty children aged </=60 months were selected. These children had no history of diarrhea, no acute respiratory infection, and no antibiotics taken for the past 4 weeks before fecal sample collection.

The nutritional status of children was based on anthropometric measures using WHO z-scores (weight for age, height for age, weight for height). To obtain an overall measure of nutritional status we calculated a cumulative z-score based on all three anthropometric measures and subdivided subjects into apparently healthy, borderline malnourished, and severely malnourished groups. Microbial composition of the 20 gut microbiomes was examined.

Abundance of different phyla. Across the 20 microbiomes, 72 % of sequenceswere assigned at phylum level. Eight phyla were present but only four were dominant – *Bacteriodetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*. These results are similar to those ofother studies. Thirty six percent of sequences were assigned at genus level. Of these, 23 genera belonged to the four dominant phyla. Calculation of rank normalized abundances of the 23 core genera showed the predominance of Prevotella across metagenomes, indicating that majority of gut bacteria in these children were similar to the Enterotype 2 community established by Arumugam and colleagues.

In 2011, a consortium of investigators combined 22 newly sequenced fecal metagenomes of individuals from

four countries with previously published data sets. They identified three robust clusters, which they called enterotypes that are nation- or continent-specific. An enterotype is a classification of living organisms based on its bacteriological ecosystem in the human gut microbiome. Enterotypes are not dictated by age, gender, body weight, or national divisions. The three enterotypes are Enterotype 1 (*Bacteroides* prevail), Enterotype2 (*Prevotella* prevail), and Enterotype3 (*Ruminococcus* prevail). While enterotypes are driven by species composition, it must be remembered that abundant molecular functions are not necessarily provided by abundant species, highlighting the importance of functional analysis. Enterotype 2, to which our subjects belonged, is predominantly distributed in Africa and is generally found in populations with high carbohydrate diet. *Bacteroides* is found where animal fat is rich in the diet. *Bacteroides plebeius*, found exclusively in Japan, metabolizes seaweeds that form a unique part of the Japanese diet.

A heat map was created, showing the normalized rank abundances of 23 genera and 8 phyla across the 20 gut microbiomes of subjects classified as healthy and malnourished. Taxa were divided into 4 groups based on similarity in their abundance patterns and labelled as G1, G2, G3, G4.Abundance of G4, predominantly comprising *Proteobacteria*, was found to increase from healthy to malnourished subjects while G1, comprising beneficial commensals, decreased with decreasing nutritional index and increased with increasing nutritional index. G2 and G3 had no significant association with nutritional index.

Among phyla, *Proteobacteria* correlated negatively while *Synergistetes* correlated positively with nutritional index. The dominant genus *Prevotella* and probiotic genera like *Lactobacillus* and *Bifidobacterium* showed no correlation with nutritional index. Several genera belonging to group 4, predominantly *Proteobacteria*, -i.e. *Escherichia, Streptococcus*, *Shigella* - were significantly overabundant in severely malnourished children compared with apparently healthy ones. Although individual genera belonging to G1 did not show any significant differences across the 3 groups, the combined abundances of all the genera belonging to G1 was significantly higher in apparently healthy children.

Genera co-occurrence networks. The symptomatic characteristics of the gut environment depend not only on the presence of specific microbial groups but also on the inherent inter-microbial co-occurrence networks present therein. Networks of co-occurring genera for the 3 groups of microbiomes were characterized by distinct network architecture.

Take for example the apparently healthy group. There are four distinct connected groups of genera – Streptococcus (G4) associated with Faecalibacterium (G1), *Enterobacter* (G1) associated with genera like *Eubacterium*, *Roseburia*, and *Dorea* (G4). There appears to be interconnection between pathogenic forms and beneficial microorganisms and perhaps beneficial microorganisms keep pathogens under check. But as nutritional status declines to borderline malnourishment, pathogenic genera come together in a single connected bar e.g. *Enterobacter* and *Shigella* groups come together. And the increasing interdependence among genera is even more pronounced for the severely malnourished group of metagenomes. It is therefore not just microbiomes per se but interconnections that differ when we look at species membership among the three cumulative nutritional indices.

Related functional categories. Sixty-four and 112 cluster orthologous genes (COG) groups were shown to have significant positive and negative correlations with the cumulative nutritional indices, respectively. COGs were referred to as Positively Correlated (PC) and Negatively Correlated (NC) COG groups.

Several functional categories associated with nutrient uptake and metabolism were over-represented or present in the PC COGs. In the NC COG group, over-represented functional categories were those associated with virulence and bacterial pathogenesis, including intracellular trafficking, secretion and vesicular transport, cell motility, inorganic ion transport and metabolism. Functionally, virulence appeared to be correlated with low nutritional index whereas functions related to metabolism of nutrients correlated positively with improved nutritional index.

Carbohydrate active enzymes (CAZymes). Among the genes identified in the human gut microbiome, those that encode CAZymes are of particular interest as these enzymes digest most of our dietary polysaccharides. The human genome encodes at most only 17 enzymes for the digestion of food glycans, specifically starch, sucrose, lactose. In contrast, the assembled mini-microbiome contains 15,882 different CAZyme genes distributed unequally between glycosyltransferases, carbohydrate esterases, glycoside hydrolases, and polysaccharidelyases genes.

We looked at the abundance patterns of CAZyme families across gut metagenomes with varying nutritional status. None of the individual CAZymes were significantly associated with cumulative nutritional index. However, when assemblages of CAZyme families having similar abundance patterns were grouped together,we found that families belonging to groups 3 and 7 had significant positive correlations with at least one nutritional index. Group 3 CAZyme families degrade complex plant carbohydrates while those in group 7 degrade mostly peptidoglycans. Group 6CAZyme families degrade complex plant carbohydrates. When the CAZyme families belonging to groups 3, 6, 7 were combined, their cumulative abundances were found to have an even stronger positive correlation with nutritional index. None of the groups had significant negative correlation with nutritional index.

I would like to enumerate the following key messages from this study:

1. There is a link between the gut microbiome and the nutritional status of children in the Indian setting where impaired nutritional status is not only due to the abundances of likely pathogenic microbial groups but also a result of depletion of several commensal genera.
2. Certain functional categories (COG) groups are positively or negatively correlated with nutritional status. Positively correlated functional groups relate to nutrients utilization while negatively correlated functional groups may initiate the infection process.

3. There are a higher number of virulence genes in children with lower nutritional index.
4. CAZyme families that degrade peptidoglycans and complex plant carbohydrates are associated with higher nutritional index.
5. Identification of distinct changes in general co-occurrence networks with progressive decrease in nutritional status of children is a key finding.
6. The gut microbiome is susceptible to modulation by disrupting certain key players in order to achieve conditions that could result in regression of the disease phenotype.
7. More comprehensive and well-designed functional studies are required for formulating a microbial basis of therapy for severe acute malnutrition.

Foods to maintain the gut microbiome and their regulation in Asia

Indigenous probiotics and immunological effects - *Ingrid S Surono, Food Technology Department, Bina Nusantara University, Indonesia/Indonesian Scientific Society for Probiotics and Prebiotics (ISSPP)*

Novel indigenous probiotic strains of *dadih*, an Indonesian traditional fermented raw buffalo milk from West Sumatra has been studied in vitro, in vivo and human studies. The process of *dadih* making involves spontaneous fermentation. The buffaloes are milked manually and the milk is collected in bamboo tubes, covered with banana leaves or plastic and kept overnight. The next day it becomes curd called *dadih*, a yogurt-like product. There is no heat application in *dadih* making but neither product failure nor food-borne illness occurs from consuming *dadih* and it is known that *dadih* has medicinal effects on health. Seventy-six lactic acid bacteria isolates of *dadih* origin from different parts of West Sumatera were screened and found 5 potential strains – 3 are *Enterococcus faecium*, and 2 are *Lactobacillus plantarum*. Among 5 strains, 2 strains – *Lactobacillus plantarum* IS-10506, and *Enterococcus faecium* IS-27526 were shown to have probiotic properties in vitro. By definition probiotics are live microorganisms which when consumed in adequate amounts confer a health effect on the host, and probiotics are strain specific. *Dadih* lactic acid bacteria might have ability to inhibit contaminants both spoilage and pathogenic bacteria.

In vitro studies

The adhesion properties of *dadih* lactic acid bacteria using mucin extracted from human feces and Caco-2 cells were examined; *Lactobacillus rhamnosus*IS-7257and *Lactobacillus reuteri* IS-27560 had high adherence to both mucus layer and Caco-2 cells.[5] Moreover, *Lactobacillus rhamnosus* showed significant inhibition towards the adhesion of *Escherichia coli* O157:H7. Overall, the adhesion properties of all dadih lactic acid bacteria strains were comparable to those of *Lactobacillus casei* Shirota and *Lactobacillus rhamnosusGG*.[5] The documented probiotic strains *Lactobacillus rhamnosus* IS-7257 *and Lactobacillus reuteri* IS-27560 were later renamed *Lactobacillus plantarum* IS-10506 and Enterococcus faeciumIS-27526.

Autoaggregation is a pre-requisite for adhesion and colonization of the gastrointestinal tract. A comparison of different *dadih* lactic acid bacteria strains showed that *Lactobacillus plantarum* IS-10506 strain had the highest autoaggregation abilities.[5]

The ability to inhibit human pathogens and displace them from intestinal mucus was assessed in vitro.[5] The most adhesive *Lactobacillus plantarum* strain was IS-10506, with 9.8% adhesion. The competitive assay between *dadih* lactic acid bacteria isolates and pathogens showed that 2 h preincubation with *L. Plantarum* IS-10506 at 37 degrees C significantly reduced pathogen adhesion to mucus. All tested *dadih* strains displaced and inhibited pathogen adhesion, but the results were strain-specific and dependent on time and pathogen strains. In general, *L. plantarum* IS-10506 showed the best ability against pathogen adhesion.[5]

The effect of probiotic administration on viable fecal microbiota was examined using a commercial probiotic *Lactobacillus casei* and *dadih* lactic acid bacteria strains *Lactobacillus plantarum* IS-10506 and *Lactobacillus plantarum* IS-20506 in a rodent model.[6] Rats given *L.plantarum* IS-10506 had significantly higher viable fecal lactic acid bacteria compared with those given commercial *L.casei* and *L. plantarum* IS-20506. On the contrary, reduction of viable fecal coliform was observed after 3 days of probiotic administration. The Sprague Dawley (SD) rats given *L.plantarum* IS-10506 showed the highest reduction in viable fecal coliform followed by *L.plantarum* IS-20506. Rats administered with *L.plantarum* IS-10506 significantly increased fecal secretory IgA after 3, and 7 days administration and after 3 days wash out period, compared with commercial strain *L. casei*. Among the probiotics administrated to the rats, *L. plantarum* IS-10506 showed the most significant effects on elevating sIgA.[6]

In another study, we examined the effect of soymilk and *L.plantarum* IS-10506 at 10^{10}cfu/day on rats infected with EPEC (enteropathogenic *E.coli*) and showed significantly increased fecal sIgA in treated animals compared with control group.[7]

Human studies

The effect of *L.plantarum* IS-10506 and zinc supplementation for 90 days on fecal sIgA and serum zinc were examined in randomized double blind placebo controlled pre post trial of four groups of Indonesian preschool children aged 12-24 months: placebo, probiotic, zinc, and a combination of probiotic and zinc. Microencapsulated *L.plantarum* IS-10506 was supplemented at a dose of 10^{10}cfu/day. Zinc was supplemented as 20 mg zinc sulfate (8 mg zinc elemental). Results showed that compared with placebo, probiotic supplementation alone significantly increased fecal sIgA while a combination of probiotic *L. plantarum* IS-10506 and zinc significantly increased fecal sIgA and improved zinc status of preschool children.[8]

In another study,[9] a randomized, placebo controlled, double-blinded pre-post trial was conducted on under 5 years children. The trial required oral consumption of probiotic *E. faecium* IS-27526 over a total period of 90 days. The 79 subject volunteers meeting the inclusive criteria were randomly assigned in consecutive way.

Among the placebo group (n=40), there were 23 girls and 17 boys, who received 1 mg maltodextrin in 125 mL commercial UHT low fat milk, and in the probiotic group (n=39), there were 17 girls and 22 boys administrated with 1 mg lyophilized *E. faecium* IS-27526 ($2.31x10^8$cfu/day) in 125 mL commercial UHT low fat milk every day for a period of 90 days. The results showed that total salivary sIgA level in placebo group was increased by 400±136 mg/mL, while in probiotic group it was increased by 919±162 mg/mL. The results indicated there was a remarkably higher augmentation of total salivary sIgA level of children in probiotic group compared with placebo group.

Based on their bodyweight, there was a significant elevation of total salivary sIgA of underweight children in probiotic group, higher than the placebo group after 90 days *E. faecium* IS-27526 (10^8cfu/day) supplementation. *E. faecium* IS-27526 enhanced total salivary sIgA level in underweight children. In addition, the mean bodyweight gain in probiotic group was1.28±0.94 kg, from 10.13±1.22 kg to 11.41±1.31 kg, while in placebo group was 0.99±0.99 kg from 10.01±1.58 kg to 11.0±1.87 kg. Taken together, *E. faecium* IS-27526 together with milk was significant in augmenting the salivary sIgA level in underweight children, and gaining weight of children with normal bodyweight. Moreover, safety has been validated in vulnerable population such as underweight young children.

In summary our studies showed the following:

- The supplementation of microencapsulated *Lactobacillus plantarum* IS-10506 at 10^{10}cfu/day for 90 days significantly increased fecal sIgA of preschool children aged 12 to 24 months. Combination of *L.plantarum* IS-10506 and zinc supplementation significantly increased both fecal sIgA and zinc status in this age group.
- Supplementation with *L.plantarum* IS-10506 had no adverse effects among apparently healthy children and immunocompromised children (undernourished).
- The gut microbiota is important for maintaining good health. Probiotics may improve nutrient absorption and enhance humoral intestinal immune response.
- Indigenous probiotics such as *Lactobacillus plantarum* IS-10506 and Enterococcus faecium IS-27526 have been exposed to surrounding microbes and are more suitable for Indonesian people.
- The recommended effective dose for *E. faecium* IS-27526 and *L. plantarum* IS-10506 at which no adverse effect is observed is 10^8 and 10^{10}cfu/day, respectively.

Regulatory challenges in the development of foods for gut health (Indonesia) – *Yusra Egayanti, National Agency for Drug and Food Control, Republic of Indonesia*

I would like to share with you our experience in developing standards for probiotics and assessing probiotics in Indonesia. According to FAO/WHO, prerequisites of a probiotic are:

- A probiotic must be alive when administered.
- A probiotic must have undergone controlled evaluation to document health benefits in the target host.
- A probiotic must be a taxonomically defined microbe or combination of microbes (genus, species and strain level).
- A probiotic must be safe for its intended use.
- Liquid milk sales in Indonesia including probiotics grew by 18% in 2010. Data on registered lactic acid bacteria (LAB)-based foods and probiotic foods in Indonesia show that (1) LAB-based foods are dominated by yogurt and only approximately 10% consist of probiotic food; (2) probiotic foods are dominated by Growing Up Milks; and (3) probiotic food products produced in Indonesia are not necessarily by national companies. Global concerns including those of Indonesia regarding use of probiotics are that (1) government regulations regarding safety assessment differ among countries, and the status of probiotics as a component in food is currently not established on an international basis; and (2) there is a need to record and analyse adverse events associated with probiotics in food and monitor long-term health benefits.

Indonesian regulations on probiotics aim to provide consumer protection and ensure fair and responsible trade. Regulations provide consumer protection by ensuring the following conditions: the safety and efficacy of probiotics; that consumers are not misled by false, ambiguous or misleading claims; that consumers have clear and accurate information on food labels; and that consumers are able to choose food properly. Our regulations take into account the following principles of good regulatory practices (GRP): transparency, relevance to science and technology development, consideration of the readiness of government, businesses, research institutes/conformity assessment bodies, and promotion of consumer awareness through effective communication.

Indonesian guidelines for evaluation of probiotic in food products

The final draft guideline for Evaluation of Probiotic in Food Products allows companies to apply for health claims for probiotic foods in Indonesia, as long as they follow the 2002 FAO/WHO Guidelines for the Evaluation of Probiotics in food (Figure 1). Companies can submit a proposal on probiotic claim to BPOM. Applications are assessed on a case by case basis. These guidelines state that:

1. The product shall fulfil the following requirements:
   - Genus, species and strain designation
   - Strain designation should not mislead consumers about the functionality of the strain
   - Minimum viable numbers of each probiotic strain at the end of the shelf life
   - The suggested serving size must deliver the effective dose of probiotics related to the health claim
   - Health claim(s)
   - Proper storage conditions
   - Corporate contact details for consumer information
   - Clinical trials among Indonesian people
2. The application should include
   - Strain specific probiotic identification
   - Functionality characterization
   - Safety and efficacy evaluation
3. Other requirements
   - Specific strain must survive the passage through the digestive tract and proliferate in the gut;

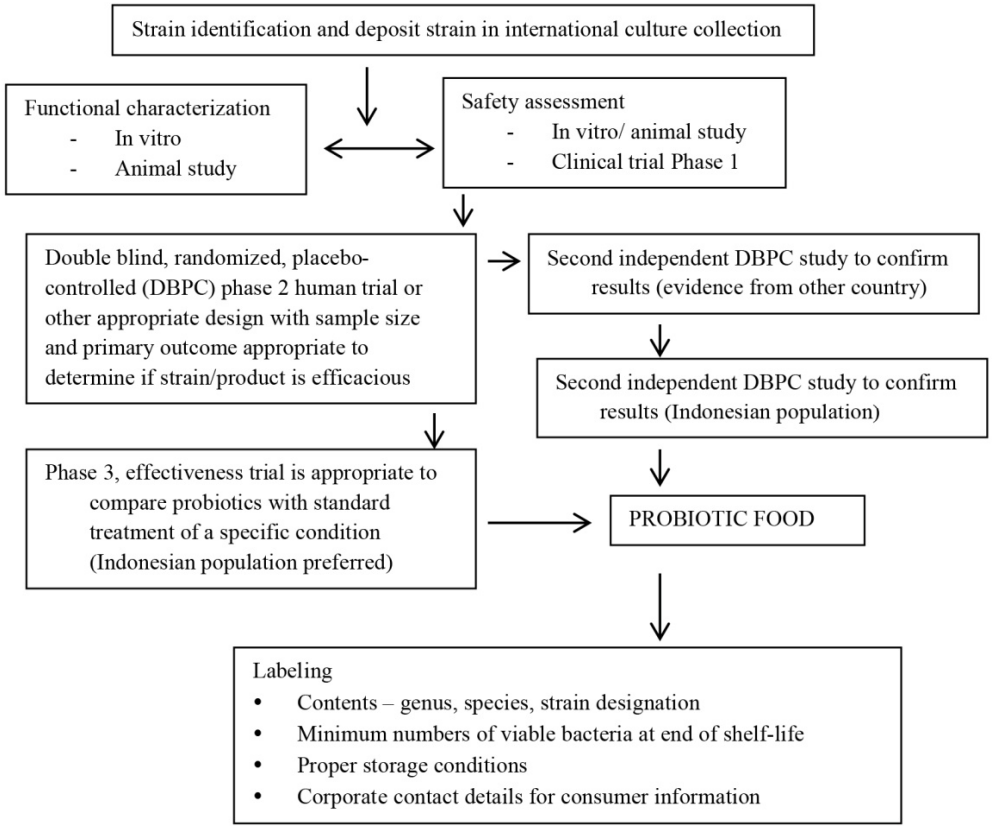

Figure 1. Summarizes the scheme for evaluation of probiotic in food products for Indonesia.

- Labelling shall include the microbial species or strain and its viable concentration;
- Claims would have to be substantiated;
- Strains are named according to the International Code of Nomenclature, be deposited in an internationally recognized culture collection;
- Strain identification shall be performed by phenotypic tests followed by genetic identification;
- Stock cultures shall be maintained under appropriate conditions and be checked periodically for strain identity and probiotic properties;
- Efficacy shall be determined by clinical trials with accepted standards of scientific quality (well-designed trials);
- Evidence based + confirmation in Indonesian population;
- Beneficial effects must be related to dosage regimens and duration of use of each individual product or strain;
- Safety considerations shall include transmission of antibiotic or drug resistance inherent in some probiotic microorganisms; the exclusion of Enterococcus strains as probiotic.

Premarket evaluation and postmarket control on probiotics in Indonesia

I will share our experience in assessing proposals from various food companies. All applications go through premarket evaluation where it is determined whether or not the company has complied with all requirements. If yes, registration approval is issued by BPOM (Indonesia National Agency of Drug and Food Control). Rejection or suspension of applications has been due to the following reasons:

- Lack of scientific evidence in human study.
- Failure to substantiate a claim based on studies with the final product type. Some proposals come to us not from the food company but from the ingredient company. It is a challenge for us how to extrapolate the study on the probiotic itself from the kind of food being applied for. This has been a problem for us.
- Inappropriate scientific evidence with respect to the proposed claim, e.g., the study was not carried out in the target group stated on the claim.
- For multi-strain probiotics: lack of data on the assessment of safety, interaction, and efficacy of multiple strains.
- Probiotic information on genus, species and strain is lacking, live microorganism (form of spore, tyndallization).
- No information on how to store the product.
- Inappropriate label.

Post market control processes include (1) food sampling and testing in distribution channels (traditional market, retailer, distribution facility, modern market); and (2) inspection of production facility. Our experiences in post market control showed lack of post market compliance with specific regulations:

- Some producers did not maintain Good Manufacturing Practices (GMP);
- Poor handling processes during storage and retailing;
- Exaggerated health claim labels;
- Labels differ from registration approval documents;
- Inappropriate advertisement;
- Expired food products;

- Broken/damaged/spoiled products.

Challenge for probiotic development

Southeast Asia region is one of mega diversity, rich in genetic resources and traditional fermented foods. Countries have a great opportunity to develop probiotics through innovation but only a few companies have the capacity to develop probiotics. Such development requires product ability to reach functionality and probiotic stability. We call for regional probiotic innovation via strong collaboration among industry, academician/scientists, and regulators.

Future actions for probiotic development in Southeast Asia particularly Indonesia include:

- Strengthening ABG (academician, business, government) partnership to develop probiotic product innovation in Indonesia;
- Developing methods (in vitro and in vivo) to evaluate the functionality and safety of probiotics;
- Following the guidelines provided by FAO/WHO as a prerequisite for development of probiotic strains;
- Following the regulatory framework for specific health claims on probiotic food labels in cases where scientific evidence exists;
- Applying good manufacturing practices (GMP) in the production of probiotic foods with quality assurance, and shelf-life conditions established;
- Setting up trial guidelines for GLP (good laboratory practice), GCP (good clinical practice), and GMP when developing probiotics for therapeutic use and health claims.

Conclusions and recommendations

1. Probiotics have received extensive attention from public, business, and research communities due to potential health benefits.
2. Indonesia's National Agency for Drug and Food Control (NADFC/ Badan POM) oversees the safety, quality, efficacy and label of the probiotic products marketed in Indonesia.
3. Probiotic foods marketed in Indonesia are mostly manufactured in Indonesia, but they are produced under international licensed probiotic strains and technology from the mother company.
4. Academician, business, and government (ABG) should strengthen interactive communication for the development of probiotics in Indonesia.
5. Growing public interest in probiotics calls for appropriate regulatory and policy action. BPOM/NADFC welcomes academician, business, and public community to discuss regarding the development of probiotics and novel function of lactic acid bacteria in Indonesia.
6. Academician and business communities should follow BPOM regulation and its guidance in early stage of development of probiotics in Indonesia to meet the requirement for product safety, quality, efficacy, and label claim.

DISCUSSION

A panel discussion at the end of the sessions looked at the gaps, opportunities, and future directions for gut microbiome research and regulations in Asia.

What microbiome research gaps need to be addressed in Asia?

Comment: I think the first gap we have to address is efficacy studies that would tell us how safe and what dose is needed in order to approve the health claim for probiotics. Maybe we can have a uniform or harmonized study design that would answer the gap. This will be a regional collaboration among Southeast Asian countries on efficacy studies on probiotics. And likewise we can start on some other gaps for characterization of the probiotics that will be used for the efficacy study.

Comment: The effects of probiotics appear to be different in different populations so harmonization might not be possible.

Comment: Indonesia is a multi-ethnic country and we found many epidemiological studies related to disease and reduction of risk factors that essentially varied from the eastern part to the western part. So it's not only about diets but also about environment. It is assumed that gut microbiota might also be different. Second, studies are very limited. In Indonesia these are dominated by studies on acute gastroenteritis in children but none of the studies are on adult or any other population. So it's really very limited data to confirm what probiotics can be effective for other populations. The government would like to practice caution in marketing probiotics in different populations, which is why probiotics are not allowed to be marketed with any claims unless they are proven in the local population.

Comment: I would like to ask the expert group - is it necessary to test the effectiveness of probiotic in every local population because this will be a real challenge for industry, specifically to take every probiotic that you wish to have that you need to test in every individual country. Going back to Europe as an example, is it the case that it actually requires a local population in the substantiation (of health claims for probiotics). Is there quite a difference in a Greek person and a normal Scandinavian person, for example, with different diets and so on. Maybe one has to go back to science and look for individual biomarkers and so on.

Comment: Are there some effects (of probiotics) that are common across populations?

Comment: We scientists have the foresight to look beyond that. The regulatory people look forward to that. Five years ago we planned a study on obese microbiome and after that, more microbiome studies were done in Europe and US. We need to have a local database and without that foresight there's no progress. What we saw yesterday from (Prof. Lee's) presentation was interesting - the idea about people originating from Africa, Italy had substantial differences in the microbiome. That kind of data becomes the basis; we do have the data in Europe already. But it don't seem in every stage that they assess everything by themselves, but it can be done. We know from some studies, for example pediatric infections like norovirus diarrhea, those probiotics that work seem to work fully, but in some other specific cases they may not be as effective. So we need the basic database for microbiome data and we also need the African microbiome data

later on. And then assessment of all this transfer among countries. I do not think that every country needs to do their own studies.

Comment: I think we need to separate politics from diet and ethnicity. What was presented, as Seppo says, shows there are certain rules people share in common in microbiome pattern. In the context of prebiotics, studies in Europe show a north-south divide in terms of the baseline intake of fructose. We have seen differences in response between Northern Europe, Central Europe, Southern Europe so dietary differences do have an impact.

Comment: I have quite extensive studies using two different probiotics in Indonesia. These probiotics are actually quite effective in European countries but only one of these probiotics is actually beneficial in our children. The study involved 500 children in prevention of rotavirus infection. The data shows strain specific reaction in different countries. Maybe Europe and Indonesia are too different. Something good to know is whether data from Malaysia can be used for Indonesia or if (data from) Indonesia can be used for Malaysia. Maybe (for countries) closer in Asia there can be a similarity.

Comment: I am a microbiologist and I look at the gut ecosystem just like a rainforest except that it's made up of microbial species. There are a few limited species, the so-called foundation species, which define the environment for everybody else to live and prosper, like the tall trees that cover the entire forest. Everything else growing underneath require a very different environment. So I think the common question is – are microbial ecosystems from different countries, different people, people with different diets – whether they have different foundation species or whether the foundation species is too low or completely gone. This is one question we still know very little about.

What do we hope to see in terms of regulations for probiotics in Asia?

Comment: This is a challenging question for such a diverse area with different dietary habits. I would certainly want to see people working together to achieve common guidelines and perhaps avoid the mistakes we have done. But where do you invest in prebiotics and probiotics for the individual microbiome? We should all be prepared to work with the appropriate professionals and communities to look into nutrition guidelines, work with medical communities on evidence-based guidelines for clinicians. I urge everybody here to join in (the discussion on) the clinical guideline across Europe especially world gastroenterology associations and pediatric associations. So working together to (develop) guidelines, regulations, and individual recommendations – I think we can achieve a lot.

Comment: My suggestion is for Asia-Pacific FDAs and their equivalents determine what the scientific consensus is. I think the role played by organizations such as ILSI is helping to develop those consensus views. In Europe, the EU is a machinery that keeps in touch with developments in scientific news. I think there's a similar opportunity in the Asia Pacific region to get together, develop that consensus, and then try and influence the regulators as they're developing the policies.

Comment: In Indonesia, we discussed recently with Ministry of Health and some experts regarding new work for Codex with respect to probiotic guidelines for Southeast Asia region. We would like to focus on the problems in Southeast Asia such as infection and nutrition transition problems including obesity. Most important is to have high quality probiotics to increase resistance to infection and improve nutrition especially among children.

Comment: I agree with the recommendation of harmonizing and working together to improve regulation of probiotics and live foods in the region. But a country like India is complex - it has 29 states with several ethnic groups and is different and diverse in several aspects including diet. I think probiotics should not bring about a divide but should be used across, whether as a (purchased) food or medicinal food of the poor. It's a big challenge for our country and the Food Standards and Safety Authority of India has found it a challenge to develop regulatory aspects for foods like probiotics. Harmonization should exist otherwise we don't come up with any guidelines, but the process also has to take into context the diversity of the people that we are talking about.

Comment: China has more unified regulations than India. But if EU have a problem of over-regulation, China may have a problem of under-regulation. So we are trying to improve to get more regulations on probiotics but I hope that we don't go too far to the other extreme, making regulations so stiff that we do not develop. Before we can harmonize regulations in global sense, I think we need a stage where we should explore the best model of regulation for each country and it will take some time before we can have global consensus.

Comment: An international conference, which can serve as a venue for discussing the topic on regulation, is the International Scientific Association of Probiotics and Prebiotics that I've been involved with for many years. I think it's about time that Asia is more represented at these meetings.

Comment: (with respect to the preceding comment) We also realized that Asia is under-represented so we have planned a satellite session on probiotics and intestinal microbiota including regulations in the next Asian Federation of Lactic Acid Bacteria meeting in Bangkok next July. One way that ILSI can be involved would be to organize a meeting where different regulatory people (here and from other countries) can discuss. The way they do things here is different and even in Europe, the US, they have a different way of regulating probiotics. It would be interesting to compare (across countries) as basically we all have the same database but the result is different.

Comment: I guess one thing I find interesting is that Europe has taken the lead in all this. It would be valuable to have a period of time where we look at the European system – what works well, hasn't worked and where much of the difficulties are. In Australia we look into European solutions, essentially guidelines, but if we can identify those difficulties many of which are being semantic in the way things are written, it will be a better place to get a good solution for Southeast Asian nations.

Comment: I would like to follow up on that argument and emphasize that there are a knowledge base spread throughout the countries. We can share that knowledge base for each country rather than each individual country

having to have an expert on each particular aspect. Since regulatory guidelines are based on the scientific evidence, each country will (have an international expert) and learn by their knowledge base and apply it. Regulatory authorities within the country will thus utilize the expertise and knowledge from outside their own county and perhaps not only look at what is specifically country-focused but the strong scientific basis for it, the scientific basis being as important as the country-specific basis.

New concepts and future directions for nutrition and the gut microbiome in Asia

Econutrition and the human microbiome

Wahlqvist[10] proposed the use of an ecosystem approach for analysing nutritional and health disorders, termed ecosystem disorders. In this approach, the integrated performance of environmental factors, food systems, and microbiological systems in combination with geographic location and societal culture influence a population's nutritional needs. A biodiverse and healthy ecosystem provides health and well-being, mediated by a healthy microbiome. Unsustainable practices that lead to environmental degradation affect the microbiome and compromise the ecosystem's ability to maintain health. One example of environmental effect on the microbiome is that of a selenium-deficient host diet (arising from deficiency in soil) on pathogenicity of the coxsackie virus that causes cardiomyopathy in infants, children, and women of child-bearing age. Here the usually benign virus became virulent due to changes in its genomic structure upon being passed through a selenium-deficient host.[11] Other examples are antibiotic resistant commensals in livestock and chemical or microbial pollutants which affect the food or water supply and consequently, the health status of populations. The concept of econutrition proposes that dietary patterns characterized by diverse food intakes and supported by healthy ecosystems is crucial in preventing ecosystem health disorders.[10] Consistent with this concept, the ILSI conference showed that a more diverse gut microbiota (which can be achieved by increased dietary diversity) is linked to better health.

Pulses in traditional Asian diets and the gut microbiome

Foods in traditional Asian diets such as legumes or pulses, particularly their fermented forms, are now recognized for their contribution to dietary diversity and healthy ecosystems. Legumes or pulses consist of plants that produce a pod with seeds inside.[12] These foods are considered important crops with significant health and nutritional benefits. Pulses also promote healthy ecosystems due to their positive effects on biodiversity, climate change, and food security.[12,13]

Effects on health

The nutritional benefits of pulses include their richer protein content than cereals, low fat and high fiber content, content of B vitamins, iron, and bioactive compounds such as phytochemicals and antioxidants.[13] Legumes and pulses act as important prebiotics. Undigestible carbohydrates in these foods (i.e., resistant starch, galactooligosaccharides) are fermented by gut bacteria, producing short chain fatty acids that are associated with reduced risk of chronic disease such a colorectal cancer. These short chain fatty acids (butyrate, acetate, propionate) inhibit transcription factor NF-KB leading to decreased secretion of inflammatory cytokines.[12] Legumes also favourably alter bowel flora to produce metabolites that alter production of gut hormones which in turn reduce appetite to promote weight loss in obesity.[12] Epidemiologic studies have shown that eating legumes can extend life by improving gut health and preventing chronic diseases.[12] The Food Habits in Later Life (FHILL) cross cultural study examined whether adherence to a Mediterranean-style diet pattern high in plant foods including legumes and low in animal foods predicted survival among long-lived cultures (i.e., age 70 years and over). Results showed that, across 5 ethnic groups studied, higher legume intake was the most predictive dietary predictor of longevity with a 7 to 8% reduction in risk of death for every 20 g increase in daily legume intake (p=0.02).[14]

Effects on the ecosystem

Pulses increase biodiversity with their soil nitrogen-fixing effects, which increase soil fertility. The presence of pulses in agricultural systems helps to maintain and/or increase vital microbial biomass and activity in the soil, providing ecosystems with greater resistance and resilience against disturbance and stress, and improving the ability of ecosystems to suppress diseases.[13] Pulses in crop rotation reduce the risks of soil erosion and their nitrogen-fixing ability reduces greenhouse gas emissions, thus providing resilience to climate change. Pulses are a cheaper source of protein and minerals than meat, have a long shelf-life, are drought-resistant and suitable for marginal environments, thus contributing to food security.[13] In this regard, FAO has declared 2016 as the International Year of Pulses in order to 1) promote increased production and development of leguminous crops due their adherence to agroecological principles for sustainable production (i.e., minimum soil disturbance, maintenance of soil cover, diversified cropping systems, increased soil fertility) and 2) to encourage greater inclusion of pulse foods in diets all over the world due to their significant health benefits and low cost.[13]

Fermented pulse products. Fermented legumes and pulses (e.g., tempe from soybeans), are traditionally consumed in Asia. Consumption of fermented pulse products increases the nutritional quality (compared with the unfermented form) but not the cost of diets, therefore providing greatest benefit to marginal populations.[15]Biochemical changes during microbial fermentation of tempe include increased content of soluble protein resulting in improved protein quality, increased content of trace minerals (Fe), vitamins (B-12, tocopherol), glucose, and isoflavones, and reduced content of lipids and phytic acid. Phytochemicals and antioxidant metabolites resulting from microbial action also impart potential health benefits including anti-diarrheal, anti-flatulent, lipid-lowering, and cancer-preventing effects.[15]

Lactase non-persistence in Asian populations and the gut microbiome

In Southeast Asia, milk consumption is low (<30

kg/capita/year) compared with that in Europe and North America (>150 kg/capita/year).[16] The low consumption of milk among Asians has been associated with lactose intolerance, defined as the inability to digest milk after infancy. At a very young age, children can produce lactase enzyme and digest lactose in mother's milk. As they mature, lactase gene expression switches off (lactase non-persistence) and only 35% of the human population can digest lactose beyond age 7 or 8.[17] Lactose intolerance (or the symptoms arising from ingestion of lactose) is traditionally viewed as a defect or health problem. Lukito et al[17] proposed that the concept of lactose intolerance be revised to that of lactase non-persistence (i.e., the natural decline in intestinal lactase to <10 μ/g of tissue which leaves adults with minimal ability to digest lactose).

Lactase non-persistence should be viewed as a natural state rather than as a defect (i.e., a default phenotype dependent on the ancestral or "wild type" version of the human lactase gene). This natural state is the norm and affects most of the global population, particularly Asians. The authors emphasized that, among lactase non-persistent populations, lactose should be considered a nutrient that improves gut health as undigested lactose in milk may enhance fermentation by colonic lactic acid bacteria (i.e., act as a prebiotic), giving rise to beneficial short chain fatty acids. Since gut bifidobacteria decline with age, milk saccharides may also have a role in preventing immune-senescence and providing a more healthful intestinal gut microbiota. In lactase non-persistent individuals, colonic microbiome lactase enables lactose fermentation to occur, allowing subjects to tolerate ≥9 to 12 g lactose (equivalent to 200 mL or 1 glass of milk) on any one occasion.[17] Consumption of traditional fermented dairy products (e.g., Indonesian *dadih* or fermented buffalo milk) would also negate the effect of lactase non-persistence. Thus it is not necessary for Asians to avoid milk or consume lactose-free products. [17]

Conclusion

The ILSI SEA Region conference showed that the gut microbiota of Asians differ from those in Europe and North America. Within the region, microbial composition differs by diet, location, and ethnic background. The composition of gut microbiota changes from birth throughout a person's life, and the mother's gut microbiota influences development of her offspring. Urbanization and economic development, dietary changes, environmental exposure, the person's health status, all affect the gut microbiome and may contribute to a shift in the region's health burden from infectious diseases to non-communicable chronic diseases. It has been recognized that the changing pattern of nutritionally related disorders and disease is a reflection of social and environmental factors, and that an ecosystem approach provides better understanding of these disorders. The ecosystem paradigm is consistent with consumption of legumes and pulses, traditionally consumed Asian foods. Within the context of Southeast Asia, the following points also need to be considered:

- Lactase non-persistence should not inhibit the consumption of dairy products among Asians, as lactose should be viewed as a nutrient that enhances gut health;
- Traditional fermented products like Tempe need to be further studied for their effects on health and to establish safe intake levels;
- Developing a variety of local fermented pulse and dairy products using indigenous microbial species would benefit Southeast Asian countries. In addition to the low cost of such foods and their contribution to improved dietary quality and diversity , indigenous microflora are better adapted to the local environment and thus more suitable for the population residing within this environment.

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AUTHOR DISCLOSURES

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